



RESEARCH ARTICLE

Acaricidal, larvacidal, and repellent activity of green synthesized silver nanoparticles against *Hyalomma dromedarii*

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ABSTRACT

We aimed at determination of acaricidal, larvacidal, and repellent activities of green synthesized silver nanoparticles (SNP) against *Hyalomma dromedarii* as one of the most common ticks in camels. SNP were green synthesized by reducing *Lupinus albus* extract through the precipitation technique. The acaricidal, larvicidal, and repellent activity of SNP against *H. dromedarii* was studied through the adult immersion test (AIT), the larval packet test (LPT), the vertical movement behavior of tick's larvae method, anti-acetylcholinesterase (AChE) activity, and oxidative enzyme activity. The green synthesized SNP displayed a spherical form with a size ranging from 25–90 nm; whereas the most distribution of particles size was reported at 50-65 nm. SNP dose-dependently ($p < 0.001$) increased the mortality rate of *H. dromedarii* adult; whereas at 16 and 32 $\mu\text{g/mL}$ completely killed the adult females. Treatment of exposure of *H. dromedarii* adult to SNP markedly ($p < 0.001$) declined the mean number, weight, and hatchability of eggs. Treatment of *H. dromedarii* larvae with SNP reduced the viability rate of larvae with the LC_{50} and LC_{90} values of 3.1 and 6.9 $\mu\text{g/mL}$, respectively. Exposure of *H. dromedarii* larvae to SNP, especially at $\frac{1}{2}$ LC_{50} and LC_{50} , markedly ($p < 0.001$) increased the oxidative stress and declined the level of antioxidant enzymes in *H. dromedarii* larvae; whereas, markedly suppressed the AChE activity of the larvae stage of *H. dromedarii* in comparison to the control group. These results showed that SNP green synthesized by *L. albus* extract had promising acaricidal, larvicidal and repellent activity against *H. dromedarii* adults and larvae as a dose-dependent response. SNP also considerably decreased the level of acetylcholinesterase and antioxidant activity and also provokes oxidative stress in *H. dromedarii* larvae. However, more investigation must be designed to clear the accurate mechanisms and the efficacy of SNP in practical use.

Keywords: Insecticide; nanoparticles; tick; larvae; control.

INTRODUCTION

Among the ectoparasites, ticks are well-known as the potent vectors for the transmission of various microbial pathogens (e.g., bacterial, protozoan, and virus) which cause several emerging and re-emerging infectious diseases of veterinary and medical importance (Boulanger *et al.*, 2019). *Hyalomma* spp. (family Ixodidae), are considered as hard-bodied ticks which play a main role in transmission of several infectious diseases with high economic loss in Saudi Arabia as well as other tropical and subtropical regions (Kumar *et al.*, 2020). *Hyalomma dromedarii* is one of the most common ticks in camels, which has an almost global distribution; whereas, it has adapted well to the very dry habitats that camels live in (Khater & Hendawy, 2014). This tick can transmit various important medical and veterinary infectious diseases, e.g., Crimean-Congo hemorrhagic fever virus, theileriosis, Q fever, and rickettsiae (ElGhali & Hassan,

2010). Existing control approaches against ticks are based on the use of chemical acaricides, e.g., carbamates, organophosphates, and pyrethroids (de la Fuente, 2018). Nevertheless, in recent years several main disadvantages have been reported for these acaricides such as reports of drug resistance emergence, deposit in foods, as well as environmental hazards (Ghosh & Nagar, 2014). Hence, it is not unexpected to declaration the urgent necessity to find eco-friendly effective agents for controlling of ticks.

Today, the desire to produce and use materials with nanometer dimensions is increasing day by day due to the interesting properties of these materials (Hasan, 2015). Metal nanoparticles have been used as attractive candidates to deliver many large and small pharmaceutical biomolecules due to their unique optical properties, catalytic properties, electrical and magnetic applications (Jamkhande *et al.*, 2019). Silver has long been used as an antimicrobial agent to treat diseases and preserve food and water (Politano *et al.*,

2013). For this reason, it can be said that the most use of silver nanoparticles (SNP) is in the medical industry (Deshmukh *et al.*, 2019). Also, silver nanoparticles showed other pharmacological properties, e.g., antimicrobial, anticancer and antioxidant (Mathur *et al.*, 2018). There are many physical and chemical methods for the synthesis of nanoparticles, but the presence of inappropriate and useless compounds is one of the disadvantages of these methods, which will be a problem, especially in the case of silver nanoparticles due to their biological and medical applications (Iravani *et al.*, 2014). The biosynthesis methods of nanoparticles using bacteria, actinomycetes, fungi and plants are very simple, low-cost, non-toxic, environmentally friendly and efficient alternative methods compared to other methods (Prasad *et al.*, 2021). The synthesis of nanoparticles is done by plant materials through the rapid reduction of metal ions. The recovery of metal ions by plant extracts is faster than any other biological system, and nanoparticles do not contain harmful compounds (Ahmad *et al.*, 2019). Therefore, nowadays, the use of plant extracts to produce nanoparticles has attracted a lot of attention. Although several investigations have been performed on the insecticidal and acaricidal effects of silver nanoparticles (SNP), however, their results were different and sometimes contradictory due to the synthesis of nanoparticles, the type of insect, and the method of application, which may affect the effectiveness of the NPs (Benelli *et al.*, 2017; Rafique *et al.*, 2017). The current investigation aimed to determine the acaricidal, larvicidal, and repellent activities of SNP against *H. dromedarii*, as one of the most prevalent ticks infesting camel in Saudi Arabia.

MATERIALS AND METHODS

Green synthesis of SNP

Lupinus albus L. seed were pre-cured in July 2022 from a shop in Riyadh, central part of Saudi Arabia. The plant was validated by a botanist and the herbarium specimen (No. 51-2022) was saved in the Department of Biological Science, Shaqra University, Saudi Arabia. By percolation technique, air-dried materials (200 g) were extracted with water for 72 h at 21°C. After filtering and evaporating of extract in a vacuum at 55°C, it was stored at -20°C (Ebrahimi *et al.*, 2017). SNP were green synthesized based on the reducing by *L. albus* extract through the precipitation technique (Albalawi *et al.*, 2021a, 2021b). To do this, AgNO₃ (1 Mm, Merck, Germany, 90 mL) was added to with *L. albus* extract (10 mL), and the combination was incubated at 21°C overnight to reduce silver ions, whereas changing the color of the extract from pale yellow to dark brown exhibited the SNP production. The obtained nanoparticles were centrifuged at 8000 rpm for 20 minutes. The sediment obtained was dried in an oven at 70°C for 5 hours. The obtained SNP were kept inside the microtube and at 4°C.

UV-vis spectroscopy analysis

Absorption spectrum of synthesized nanoparticles was performed by a UV-vis spectrophotometer to investigate the alteration of the Ag ions to SNP. Briefly, NPs solution (300 µL) was diluted with normal saline (3 mL) and was studied by UV-vis spectrum analysis engaging a spectrophotometer device (Shimadzu UV2550, Japan) in the range of 300–700 nm.

Physical characterization of SNP

The size and shape of the green synthesized SNP was evaluated by a scanning electron microscope (SEM, Mira3, Czech- 15 kV, a magnification of 10x, and a resolution of 1 nm) and using a Dynamic light scattering (DLS) device (Zeta sizer, UK, Malvern).

X-ray diffraction (XRD) analysis

The presence of silver in green synthesized SNP and their the crystal organization was assessed by assessing the Ka-ray source of a copper

lamp with a wavelength of X beams in $\lambda = 1.54 \text{ \AA}$ by a XRD device model 2000 APD, Italy.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR analysis was done to identify the biomolecules responsible as coating agent of the synthesized nanoparticles. In brief, the SNP powder was mixed with potassium bromide to yield tablets and studied using a device (Tensor27, Germany).

Collecting the ticks

Adult females of *H. dromedarii* (500 mg) were collected from naturally infested camel in rural regions of Riyadh, Saudi Arabia. Then, all the ticks with the same weight range were selected in Petri dishes. Petri dishes were examined under a stereomicroscope and the species of the tick were identified in the laboratory according to the standard method (Estrada Peña *et al.*, 2004). They were washed with normal saline and dried; whereas several ticks were used for adult immersion analysis, and the rest were reserved at 27°C and 75–80% relative humidity to shed eggs and larvae.

Adult Immersion Test (AIT)

Briefly, nine groups of adult ticks (10 ticks per each) separately immersed with SNP at 0.312-200 µg/ml, normal saline (negative control), and deltamethrin (positive control) for 5 min at 21°C. AIT was performed as the triplicate. After drying the ticks, they were put in a Petri dish and incubated at standard conditions until oviposition was complete (Gazim *et al.*, 2011). After 14 days, the number of ticks leaving eggs and the weight of the collected eggs was recorded. To determine the hatchability rate, eggs were moved to tubes and kept for 21 days at standard conditions. Additionally, the lethal concentration 50 (LC50) and LC90 values of SNP were determined using the Probit test.

Larvicidal activity of SNP

The larval packet test (LPT) was used to assess the larvicidal effects of SNP (Matos *et al.*, 2019). Briefly, nine groups of larvae (15 larvae per each) at 10 days-old were separately put in the center of filter papers (7×7 cm) and SNP at 0.312-200 µg/ml were added to them and closed to make packets. Followed by one-day incubation in standard conditions, packets were tested to determine the rate of larvae viability, while larvae with no motility or movement were recorded as dead larvae.

Repellent activity of SNP

The vertical larval motility behavior approach was used to assess the repellent effects of SNP as described by Wanzala *et al.* (2004). To do this, a device with two aluminum rods (0.7×15 cm) with filter paper (7×7cm) were soaked with SNP at 0.625–40 µg/ml, normal saline, and DEET (N,N-diethyl- 3-methylbenzamide, 7.5%). The soaked paper was cut on one rod, on the other rod. Next, 10-day larvae (n=30) were placed at the base of each rod and monitored for 60–240 min. Larvae found on the upper and lower end of the soaked filter paper were measured as not repelled and repelled, respectively.

Oxidative enzyme activity

Initially, larvae stage was exposed to SNP at 1/3 LC₅₀, ½ LC₅₀, and LC₅₀, then the larvae homogenate was obtained and the level of lipid peroxidation (malondialdehyde, MDA) was determined colorimetrically (Ohkawa *et al.*, 1979; Alanazi *et al.*, 2022). The level of glutathione-S-transferase (GST) as an antioxidant enzyme was also studied on the larvae homogenate acquired, based on the reaction of 5,5'-dithiobis (2-nitrobenzoic acid, Sigma-Aldrich, St. Louis, MO, USA) with GST as described elsewhere (Beutler *et al.*, 1963; Alanazi *et al.*, 2022).

Anti-acetylcholinesterase (AChE) activity

In order to investigate the AChE effects, nine groups of larvae (15 larvae per each) were separately exposed to SNP (1/3 LC50, ½ LC50, and LC50), deltamethrin (1mL/L, positive control), and normal saline (negative control), and were macerated by means of a mortar and grinder for 10 min in a mixture of sodium phosphate buffer (100 mM, pH 7.0), Triton X- 100, and protease inhibitor (at a ratio of 1 to 5 larva weight: buffer volume). The achieved combination was incubated for 30 min at the refractor. After centrifuging the combination at 7000 g for 25 min, the upper part was obtained and reserved at 4 C (dos Santos Cardoso *et al.*, 2020). The level of inhibition of the AChE enzyme was determined according to the method described elsewhere.

Statistical analysis

All experiments were carried out in triplicate to increase the reliability of the results. The acquired data was analyzed by SPSS software ver. 26.0, whereas, one-way ANOVA and a t-test were applied to compare the tested groups. $P < 0.05$ was finally measured as significant difference.

RESULTS AND DISCUSSION

The absorption peak of nanoparticles obtained from the Vis-UV was observed at 448 nm, which represented the presence of silver nanoparticles (Figure 1A). SEM analysis also showed that the green

synthesized SNP displayed a spherical form with a size ranging from 25–90 nm; whereas the most distribution of particles size was reported at 50–65 nm (Figure 1B). The findings of XRD analysis showed the presence of a diffraction peak at 27.5°, 31°, 37.8°, and 42.9°, corresponds to the 111, 200, 220, and 311 a.n, respectively, represented the cubic structure of silver crystals, (Figure 1C). The results of FTIR revealed that the extract reduced the of silver ions (AgNO_3); hence, it might be applied as coverings agent for nanoparticles. The bands at 3406, 3082, 1781, 1653, 1508, 1392, and 1198 cm^{-1} are related to the reaction of plant extract with silver ions, the O–H stretching of alcohol and phenol, C–H stretching of the aliphatic group, C–O stretching of ester carbonyl, C–C stretching of the aromatic ring, and C–O stretching of ester, respectively (Figure 1D). Reviews showed the various plants, e.g., *Ananas comosus*, *Argemone mexicana*, *Malus domestica*, *Polyalthia longifolia*, *Ocimum sanctum*, *Jatropha curcas*, *Acorus calamus*, and *Chenopodium album* have been used for the green synthesis of SNP; whereas they produced SNP in a spherical form with different sizes ranging from 2–200 nm depending on extract concentration and synthesis methods (Rafique *et al.*, 2017).

The AIT assay revealed that SNP dose-dependently ($P < 0.001$) increased the mortality rate of *H. dromedarii* adult; whereas at 16 and 32 $\mu\text{g}/\text{mL}$ completely killed the adult females (Figure 2A). The obtained LC_{50} and LC_{90} values for SNP were 6.3 and 12.8 $\mu\text{g}/\text{mL}$, respectively. The findings also showed that after treatment of *H. dromedarii* adults to different concentrations of SNP, the mean

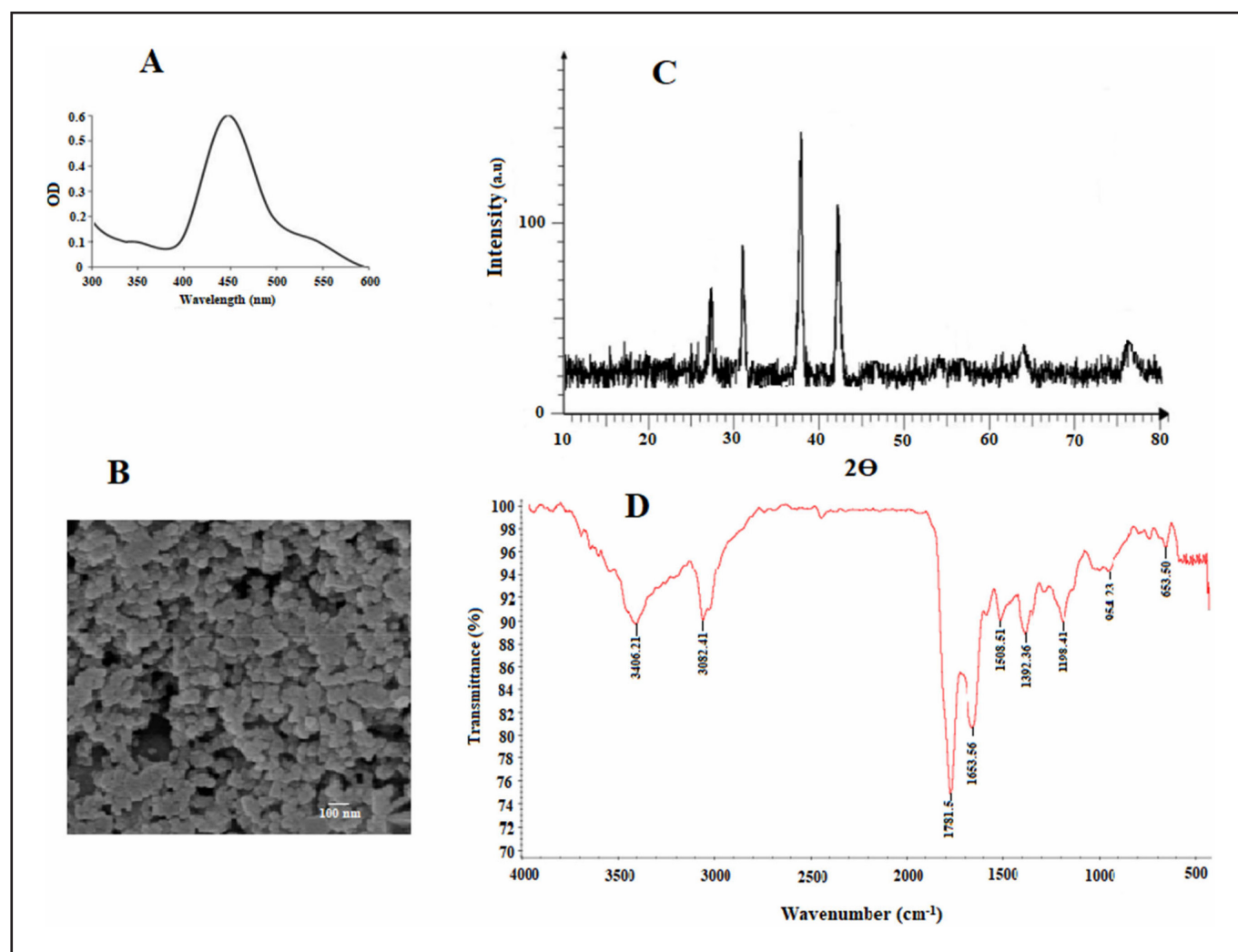


Figure 1. UV-Vis spectroscopic analysis (A), scanning electron microscope (B), X-ray diffraction (XRD) analysis (C), and fourier transform infrared spectroscopy (FTIR) analysis (D) of the green synthesized silver nanoparticles.

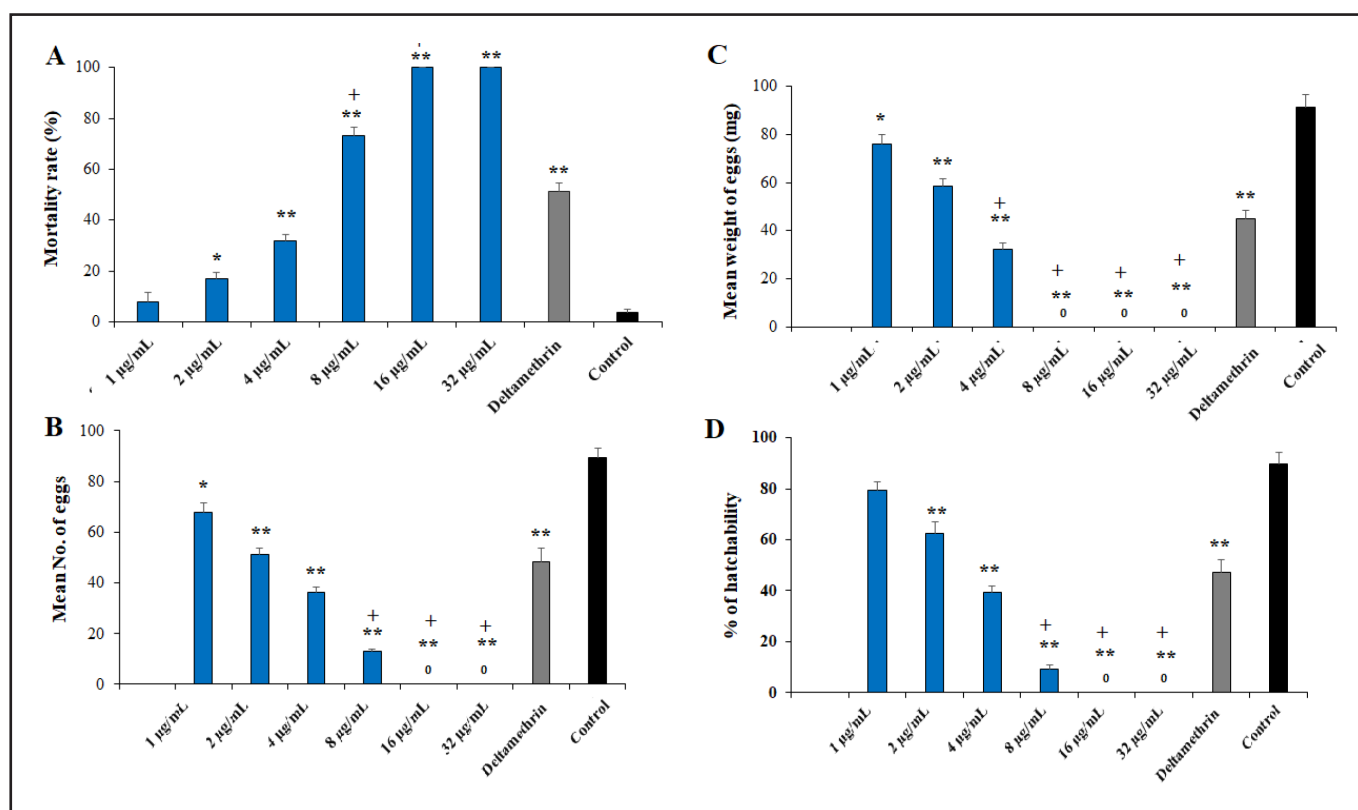


Figure 2. Effects of the green synthesized silver nanoparticles on mortality rate (A) on adult females of *Hyalomma dromedarii* as well as weight (B) number (C), and hatchability (D) of eggs. (n = 3). * p < 0.001 compared with control group; + p < 0.001 compared with deltamethrin.

number (Figure 2B), weight (Figure 2C), and hatchability (Figure 2D) of eggs were dose-dependently declined when compared to the control group. The results showed that after 24h exposure of *H. dromedarii* larvae with different concentrations of SNP, the viability rate of larvae was considerably ($p < 0.001$) declined; whereas, at 8, 16, and 32 µg/mL killed the 100% of larvae. The obtained LC_{50} and LC_{90} values for SNP were 3.1 and 6.9 µl/mL, respectively (Figure 3). Figure 4 showed that SNP showed the repellent activity against *H. dromedarii* larvae in a dose-dependent response ($p < 0.01$). The maximum activity was reported at 16, and 32 µg/mL with complete repellent activity by 60 and 120 min of exposure, respectively ($p < 0.001$).

It has been proven that ticks during blood meals are displayed significant amount of reactive oxygen species (ROS) because of presence of some blood elements which can provoke oxidative stress and subsequently damage ticks (Hernandez *et al.*, 2022). A normal harmony amongst production of ROS production (oxidative stress) and its deactivation (antioxidant) strengthens survival and ticks abilities and inhibits the effects of acaricidal agents. Thus, a potent acaricidal agent by disturbing this balance through decreasing the antioxidant activities and increasing oxidative stress can cause serious damage to the critical organisms of ticks (Hernandez *et al.*, 2022). We found that after the exposure of *H. dromedarii* larvae to SNP, especially at $\frac{1}{2}$ LC_{50} and LC_{50} , markedly ($p < 0.001$) increased the MDA level; on the other hand, ($p < 0.001$) declined the level of GST in *H. dromedarii* larvae (Figure 5).

Acaricidal largely, display toxicity through hindering the AChE, that reduces ACh, a principal neurotransmitter in the tick central nervous system (Mladenovic *et al.*, 2018). Although the accurate acaricidal mechanisms of AChE are not yet clearly understood, but AChE driven inhibitors disrupt the structure of autonomic ganglia, neuromuscular, essential effector modulated by ACh (Mladenovic *et al.*, 2018). Therefore, the discovery of a new acaricidal agent, especially with the inhibitory activity of ACh, seems very

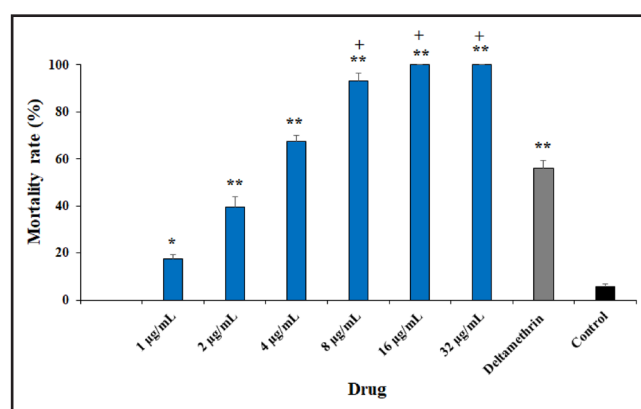


Figure 3. Larvicidal effects of the green synthesized silver nanoparticles on *Hyalomma dromedarii* larvae. (n = 3). * p < 0.05 significant difference with control group; ** p < 0.001 compared with control group; + p < 0.001 significant difference with deltamethrin.

necessary to control ticks. Figure 6 displayed the effects of SNP on the AChE inhibition in the *H. dromedarii* larvae. The results showed that the SNP mainly at $\frac{1}{2}$ LC_{50} and LC_{50} concentrations markedly suppressed the AChE activity of the larvae stage of *H. dromedarii* in comparison to the control group.

Currently, the principle strategies for controlling ticks are utilizing chemical agents that are associated with various side effects, e.g., high toxicity, environment hazard, and the emergence of resistance to these agents (de la Fuente, 2018). In recent years, the acaricidal effects of several metal nanoparticles (e.g., silver, zinc, titanium, and gold) against various hard and soft ticks have been reported (Benelli *et al.*, 2017; Rafique *et al.*, 2017). Nevertheless, their results have been diverse and often inconsistent, which could

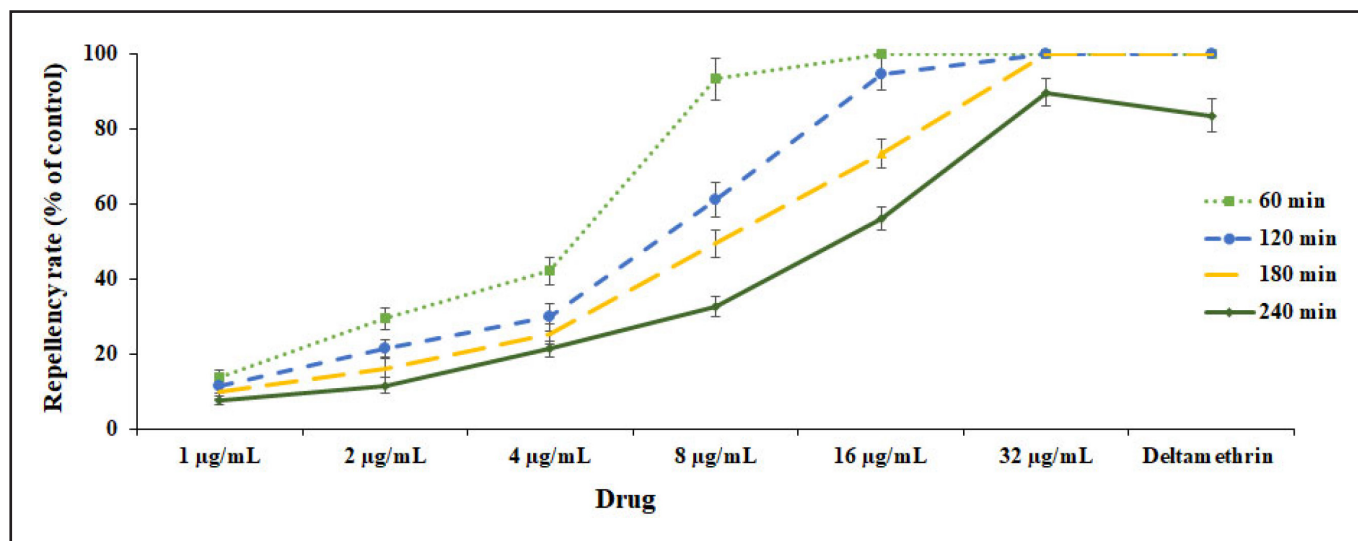


Figure 4. Repellent activity of the green synthesized silver nanoparticles on *Hyalomma dromedarii* larvae. (n = 3). DEET: (N,N-diethyl-3-methylbenzamide, 7.5%).

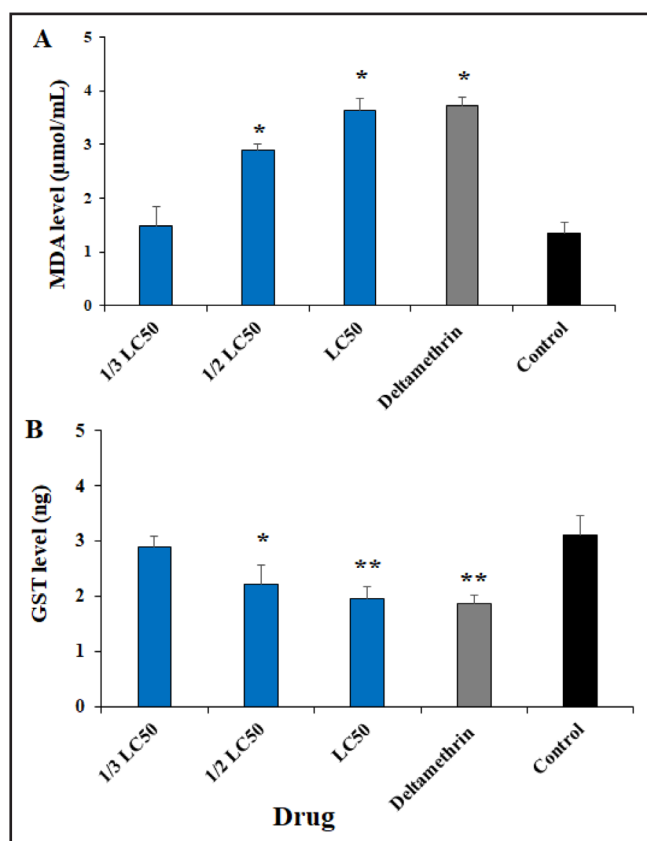


Figure 5. The effect of the green synthesized silver nanoparticles on the oxidative enzyme activity in *Hyalomma dromedarii* larvae. LC₅₀: the lethal concentration 50; MDA (A): malondialdehyd; GST (B): Glutathione S-transferases (n = 3). * p < 0.05 compared with control group; ** p < 0.001 compared with control group.

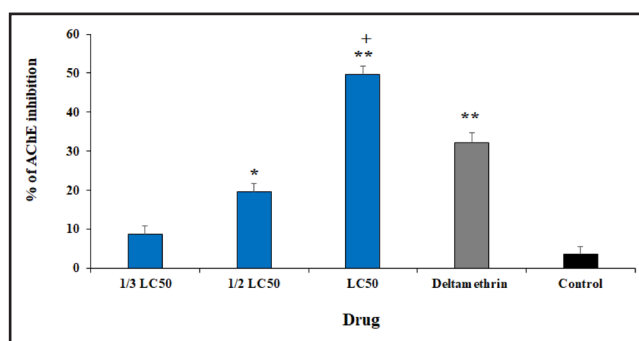


Figure 6. Anti-acetylcholinesterase (AChE) activity of the green synthesized silver nanoparticles on *Hyalomma dromedarii* larvae. LC₅₀: the lethal concentration 50. (n = 3). * p < 0.05 significant difference with control group; ** p < 0.001 compared with control group; + p < 0.001 significant difference with deltamethrin.

affect their efficacy because of the type of synthesis, herb used, tick type, and the method of assay (Benelli *et al.*, 2017; Rafique *et al.*, 2017). For example, Zahir and Rahuman (2012) showed that SNP green synthesized by *Euphorbia prostrata* aqueous leaf extracts showed the potent acaricidal effects against *Haemaphysalis bispinosa* adult cattle tick with LC₅₀ and LC₉₀ value of 2.3 and 9.03 mg/L, respectively. Jayaseelan and Rahuman (2012) have reported the considerable acaricidal effects SNP green synthesized by *Ocimum canum* aqueous leaf extract on the larvae stage of *H. anaticum* and *H. isaaci* with LC₅₀ value of 15.31 and 13.85 mg/L, respectively; whereas, these values for LC₉₀ were 62.41 and 48.86 mg/L, respectively. In a study Rajakumar *et al.* (2011), SNP green synthesized by *Manilkara zapota* extract showed potent acaricidal effects against *Rhipicephalus microplus* were with the LC₅₀ value of 3.44 mg/L respectively. Concerning the mechanisms of

insecticide action of metal nanoparticles, previous studies showed that these nanoparticles displayed the insecticide effects through reducing activity of antioxidant enzymes and subsequently provoke oxidative stress and cell death, suppressing the acetylcholinesterase activity and CYP450 isoenzymes, inhibition of trypsin and later development and reproductive disorder, reducing membrane permeability, destruction vital organelle and enzymes, inhibiting the synthesis of proteins and gonadotrophins, and induction of dehydration of insects (Benelli, 2018).

CONCLUSION

These results showed that SNP green synthesized by *L. albus* extract had promising acaricidal, larvicidal and repellent activity against *H. dromedarii* adults and larvae as a dose-dependent response. SNP also considerably decreased the level of acetylcholinesterase and antioxidant activity and also provokes oxidative stress in *H. dromedarii* larvae. However, more investigation must be designed to clear the accurate mechanisms and the efficacy of SNP in practical use.

Ethical statement

This work was permitted by the Ethics Committee at Almaarefa University, Saudi Arabia (IRB23-030).

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Disclosure

The author declares that they have no competing interests

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